REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Pursuant to 37 CFR § 1.121, attached as an appendix is a version of the amendments with markings to show changes that have been made.

Transfer of genes into plants is an approach being used with increasing frequency to provide useful and advantageous characteristics to crop and ornamental plants that would be difficult or impossible by traditional breeding methods. Transgenic traits can provide the capacity to synthesize specific compounds including vaccines, antibodies, pharmaceutical peptides, plastic, or industrial enzymes or provide improved physical characteristics such as modified fruit ripening, altered fiber properties, enhanced nutrient or dietary fiber content, herbicide resistance, floral color, or better flavor. Other introduced traits are intended to overcome or minimize particular agricultural problems, such as environmental stress, or attack by specific pathogens or pests that prevent maximum yields from being obtained. Transgenic traits that have been commercialized to date have had very specific and limited functions. Many other transgenic traits currently being developed for commercialization or being considered for introduction into crops are similarly limited or specific in their function.

Environmental factors are an important constraint on the yields obtained from transgenic as well as non-transgenic crops. Losses in productivity due to disease and damage caused by pathogens and pests can prevent the full benefit of a transgenic trait from being realized. Since many transgenic traits have no effect on disease or pest resistance, transgenic plants are typically just as susceptible to loss and damage as non-transgenic plants. Transgenic traits designed to confer resistance to pests or disease are, in general, limited in scope — i.e., they are effective only against specific pests or diseases. Such transgenic plants are as vulnerable to non-target pests and diseases as non-transgenic plants. Moreover, the process of introducing a transgenic trait can on occasion result in a crop plant becoming more susceptible to a particular disease. This was observed for some varieties of insect resistant transgenic cotton that lost resistance to a particular fungal pathogen.

Genetically determined inherent growth characteristics of any transgenic plant impose an additional limitation on the potential for benefit to be gained. Transgenic traits being developed for commercialization or that have been commercialized to date do not

affect plant growth properties, so efficacy of the traits is restricted by an upper limit on growth even under ideal growing conditions. In some cases it has been observed that the introduction of a transgene conferring a value-added trait can actually cause a reduction in yield. Such a reduction in yield is known as a yield penalty. Yield penalties are tolerated when the value-added trait results in a net economic gain; however, reducing or eliminating the yield penalty would be a clear benefit.

A practical constraint on realizing the maximal benefit from transgenic traits is imposed by the length of time required to develop a transgenic crop to the commercial stage. By the time a transgenic line reaches commercialization, the germplasm used as the starting material may be five or more years old and be at a disadvantage in terms of yield or resistance to specific diseases or pests relative to new germplasms developed in the intervening years. Therefore, it would be desirable to provide an approach that would maximize the benefits of a value-added trait, overcome the yield penalty caused by introduction of a value-added trait, and more rapidly develop a transgenic crop or ornamental lines. To achieve these objectives using existing methods or strategies would be excessively time consuming, technically complex, and without any guarantee of success.

A conventional breeding program is one approach that could be chosen to attempt to obtain a genetic background exhibiting enhanced growth and resistance to diseases and pests into which transgenic traits could be introduced. Unfortunately, achieving even marginal improvements in any one of these characteristics by classical breeding has become increasingly difficult and time consuming as the remaining amount of untapped genetic resources available within a given crop species becomes smaller. There is also no guarantee that this approach is feasible since it is unknown whether achieving useful improvements in all these characteristics simultaneously is possible by conventional breeding.

An alternate approach, at least in principle, would be to introduce into plants, in addition to a gene conferring a desired value-added trait, an array of genes each with a specific resistance or growth enhancement trait to provide an umbrella of resistance and yield improvement effects. A large number of genes have been identified that encode proteins with potential to provide resistance to specific types or classes of pathogens if expressed in transgenic plants. In principle, assembling multiple resistance genes in a transgenic plant could confer resistance to a broad range of pathogens. Such resistance genes, however, would not alter the inherent growth characteristics of the plants. Candidate genes that would serve to enhance overall growth and yield in concert with resistance genes are not obvious. Successfully producing transgenic crops that express arrays of transgenes would be

technically complex and require even longer development times than are already needed for generating transgenic plants with a single transgene. Introduction of arrays of transgenes into the same crop plant is an approach yet to be proven in practice.

The use of chemical supplements, including fertilizers and pesticides, to enhance realization of value-added traits is also undesirable due to direct and lingering environmental impact which the chemical supplements can have on water supplies and other organisms in the food chain.

The present invention is directed to overcoming these and other deficiencies in the art.

The rejection of claims 1-21 under 35 U.S.C. § 112 (first paragraph) for lack of written descriptive support in the specification is respectfully traversed.

The U.S. Patent and Trademark Office ("PTO") has asserted at page 3 of the outstanding office action that the claims are broadly drawn to a method of topical application of any of a multitude of hypersensitive response elicitor proteins from any source to plants transformed with any of a multitude of DNA molecules that impart a transgenic trait to a plant. While the PTO acknowledges that the specification does describe the topical application of the hypersensitive response elicitor HrpN from *Erwinia amylovora* to Bt-transgenic corn, the PTO suggests that the specification fails to describe (i) additional hypersensitive response elicitors or (ii) other plants comprising other transgenic traits. Applicants respectfully disagree for the reasons set forth below.

Applicants submit that page 7, line 25 through page 26, line 8 of the present application contains detailed descriptions of more than seven exemplary hypersensitive response elicitors from various plant pathogens. As demonstrated in the accompanying Declaration of Ernest J. De Rocher Under 37 C.F.R. § 1.132 ("De Rocher Declaration"), hypersensitive response elicitor proteins and polypeptides are an art-recognized class of compounds and, therefore, results obtained with one hypersensitive response elicitor would be expected for other hypersensitive response elicitor proteins or polypeptides.

In plants, the hypersensitive response phenomenon results from an incompatible interaction between plant pathogens and non-host plants (De Rocher Declaration ¶ 5). These types of interactions involve, for example, a bacterial plant pathogen attempting to infect a host plant, and the host plant preventing proliferation of the pathogen by the collapse and death, or necrosis, of plant leaf cells at the site of infection (Id.). This is distinct from a compatible interaction between a bacterial plant pathogen and a host plant in

which the bacteria is capable of proliferation, resulting in the spread of the pathogen throughout the plant and the manifestation of disease symptoms (Id.).

Hypersensitive response elicitors within a given genus are often homologous to elicitors from different pathogenic species and strains of the same genus (De Rocher Declaration ¶ 6). For example, homologs of hypersensitive response elicitors from *Erwinia amylovora* and *Pseudomonas syringae* have been identified in different bacteria species and strains from the genera *Erwinia* and *Pseudomonas*, respectively (Id.).

In addition, numerous reported studies confirm that a gene encoding a hypersensitive response elicitor from a particular source genus can be used to isolate a corresponding hypersensitive response elicitor gene from different species and strains of that same genus (De Rocher Declaration ¶ 7). For example, the *Erwinia amylovora* hypersensitive response elicitor encoding gene was used as a probe to isolate, clone, and sequence the gene encoding the *Erwinia chrysanthemi* hypersensitive response elicitor (Id.). Likewise, the gene encoding the *Erwinia carotovora* hypersensitive response elicitor has be isolated, sequenced, and cloned by using the *Erwinia chrysanthemi* hypersensitive response elicitor encoding gene to probe the genomic library of *Erwinia carotovora* (De Rocher Declaration ¶ 8); and the gene encoding the hypersensitive response elicitor of *Erwinia amylovora* has also been used as a probe to isolate and clone the gene encoding the hypersensitive response elicitor of *Erwinia stewartii* (De Rocher Declaration ¶ 9). It was found that antibodies raised against the hypersensitive response elicitor of *Erwinia stewartii* cross-reacted with the hypersensitive response elicitor of *Erwinia amylovora* (Id.).

Similar findings were reported for hypersensitive response elicitors from the genus *Pseudomonas* (De Rocher Declaration ¶ 10). An internal fragment of the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae* (i.e., *hrpZ*) was used to identify and isolate the hypersensitive response elicitors from *P. syringae* pv. *glycinea* and *P. syringae* pv. *tomato* (Id.). Significant amino acid sequence similarities were identified between the various *Pseudomonas syringae* elicitors (Id.).

The genes encoding hypersensitive response elicitors are positioned within the *hrp* gene cluster or proximate to the *hrp* gene cluster in *hrp* regulons (De Rocher Declaration ¶ 11). For example, *hrpN* from *Erwinia amylovora* was located within the *hrp* gene cluster, as was *hrpZ* from *Pseudomonas syringae* (Id.). The *popA* gene, encoding a hypersensitive response elicitor from *Pseudomonas* (now *Ralstonia*) *solanacearum*, was located on the left flank of the *hrp* gene cluster within a *hrp* regulon (Id.). Similar to the *popA* gene, *hreX*, the

gene encoding a hypersensitive response elicitor from *Xanthomonas campestris*, was located on the left flank of the *hrp* gene cluster (Id.).

The characteristics that distinguish hypersensitive response elicitors as a distinct class of molecules are clearly apparent when considering the different elicitors' secretion mechanisms, regulation, biochemical characteristics, and biological activities (De Rocher Declaration ¶ 12). Substantially all hypersensitive response elicitors identified have been shown to be secreted through the type III, *hrp* dependent secretion pathway (De Rocher Declaration ¶ 13). The type III secretion pathway is a highly conserved and unique mechanism for the delivery of pathogenicity related molecules in gram-negative bacteria (Id.). The *hrp* gene cluster is largely composed of components of the type III secretion system (Id.). Regulation of the genes encoding the *hrp* gene cluster, and subsequently the genes encoding the components of the type III secretion system and hypersensitive response elicitors, is controlled by environmental factors (De Rocher Declaration ¶ 14). Specifically, transcriptional expression of these genes is induced under conditions that mimic the plant apoplast, such as low concentrations of carbon and nitrogen, low temperature, and low pH (Id.). Biochemically, hypersensitive response elicitors have a number of common characteristics (De Rocher Declaration ¶ 15). These include being glycine rich, heat stable, hydrophilic, lacking of an N-terminal signal sequence, and susceptible to proteolysis (Id.). In addition, hypersensitive response elicitors share a unique secondary structure that has been associated with these elicitors' distinct biological activities (described below) (De Rocher Declaration ¶ 16). The structure has two primary components, an alpha helix unit and a relaxed acidic unit having a sheet or random turn structure (Id.). In the absence of one or both of these components, hypersensitive response elicitation does not occur (Id.).

Hypersensitive response elicitor proteins and polypeptides also share a unique ability to impart specific plant responses (De Rocher Declaration ¶¶ 17-34). These plant responses include disease resistance, growth enhancement, and plant stress resistance to a variety of plant environmental stressors (Id.). The responses can be achieved through topical application of the hypersensitive response elicitor to a plant (Id.). Theses plant responses have been observed in both monocot and dicot plants, and in a variety of species ranging from tomato, tobacco, cucumber, potato, raspberry, Arabidopsis, corn, lima bean, cotton, to various ornamentals (Id.).

Given that the present application describes a number of hypersensitive response elicitor proteins and methods for the application thereof to transgenic plants (page 26, lines 9-19; page 29, line 20 to page 32, line 2) as presently claimed, and given that one or

ordinary skill in the art would understand that results achieved with one hypersensitive response elicitor would be expected with other members of the art-recognized class of hypersensitive response elicitors, applicants submit that the present application provides written descriptive support for the entire art-recognized class of hypersensitive response elicitors as presently claimed. The PTO has cited no basis for suggesting that the HrpN of *Erwinia amylovora* should be distinguished from other members of the art-recognized class of hypersensitive response elicitor proteins.

With respect to the second basis for rejection, applicants submit that the specification clearly recites that, "[t]he transgene or DNA molecule conferring a trait can be any DNA molecule that confers a value-added trait to a transgenic plant" (page 33, lines 1-2). The present invention is therefore clearly directed towards transgenic plants harboring a value-added trait, and not towards transgenic plants harboring any transgenic trait as suggested by the PTO. Moreover, at page 33, line 2 through page 36, Table 1, the present application identifies numerous examples of transgenic plants transformed with transgenes conferring value-added traits. Table 1, in particular, describes value-added traits conferred by transgenes and incorporates by reference the patents and literature that describes the transgenic plants and uses thereof. In addition, page 36, line 2 through page 44, line 24 of the specification describes specific methods for producing such transgenic plants.

Because hypersensitive response elicitor proteins are known to induce broad disease resistance, growth enhancement, and stress resistance, one or ordinary skill in the art would understand — given applicants demonstration with harpin$_{Ea}$ applied to Bt transformed corn and the substantial disclosure of both other hypersensitive response elicitors and transgenic plants possessing value-added traits — that applicants were in possession of the genus as presently claimed.

For all these reasons, the rejection of claims 1-21 for lack of written descriptive support is improper and should therefore be withdrawn.

The rejection of claims 1-21 under 35 U.S.C. § 112 (first paragraph) for lack of enablement is respectfully traversed.

The PTO has taken the position that the specification does not enable the use of hypersensitive response elicitors other than harpin$_{Ea}$ for topical application to plants possessing a transgenic trait other than Bt-conferred pest resistance. For the reasons set forth below, applicants respectfully disagree.

With respect to the rejection concerning the use of hypersensitive response elicitors other than harpin$_{Ea}$, applicants submit that, for the reasons noted above, results obtained from one hypersensitive response elicitor protein or polypeptide would also be expected from other hypersensitive response elicitor protein or polypeptide. Therefore, one of ordinary skill in the art would be fully able to use other members of the art-recognized class of hypersensitive response elicitors.

In any event, as further support that the present application enables one of ordinary skill in the art to practice the full scope of the claimed invention, applicants refer to paragraphs 35 through 38 of the De Rocher Declaration. As demonstrated in the De Rocher Declaration, HrpN from *Erwinia amylovora* and HreX, a hypersensitive repose elicitor from *Xanthomonas campestris*, are both capable of increasing the efficacy of an herbicide-resistance trait in an herbicide-resistant transgenic corn variety, thereby maximizing the benefit of the value added trait conferred by the transgene to the transgenic plant (Id.).

With respect to the rejection concerning transgenic traits other than Bt-conferred pest resistance, applicants direct the PTO to page 33, lines 1 through 12 of the present application, wherein the specification clearly delineates the transgenic plants which can be treated in accordance with the present invention. The specification recites that "transgenes or DNA molecules conferring a trait can be any DNA molecule that confers a value-added trait to a transgenic plant" (page 33, lines 1-2), and furthermore that "[t]he transgenes conferring a value-added trait can encode either a transcript (sense or antisense) or a protein or polypeptide which is different from the hypersensitive response elicitor protein or polypeptide" (Id. at lines 10-12). The specification therefore clearly delineates that the invention is not directed towards transgenes conferring any trait whatsoever, but instead only to those transgenes that (1) confer a value-added trait and (2) do not encode a hypersensitive response elicitor protein or polypeptide.

As noted above, the specification provides ample description of transgenic plants transformed with transgenes conferring value-added traits. As the PTO has noted, Examples 6, 7, 8, and 10 of the present application further describe transgenic plants possessing value-added traits that can be treated in accordance with the presently claimed invention, including herbicide resistant transgenic crops, insect resistant transgenic crops, enhanced nutritional value transgenic crops, and pathogen resistant transgenic crops (pages 52 through 54, and 55 through 56). The procedures, materials, and considerations that one skilled in the art would take into consideration in producing a transgenic plant (transformed

with a transgene that confers a value-added trait) have been provided at page 36, line 1 through page 44, line 24 of the present application.

Paragraph 39 of the De Rocher Declaration provides further evidence that transgenes can confer both a value-added trait and a deleterious effect to the transgenic plant. As cited in the De Rocher Declaration, Elmore et al., "Glyphosate-Resistant Soybean Cultivars Yields Compared With Sister Lines," Agronomy Journal 93:408-412 (2001) (copy attached as Exhibit 18 to De Rocher Declaration) reports a study in which transgenic glyphosate-resistant soybean and non-transgenic soybean sister lines were evaluated in four locations over a two year period for differences in yields (Id.). The glyphosate resistant soybean lines yielded approximately 5% less (kg/ha) than the non-transgenic soybean sister lines (Id.).

From the foregoing, it should be appreciated that one of ordinary skill in the art would be fully able to prepare a transgenic plant whose transgene confers a value-added trait and then treat the transgenic plant with topical application of a hypersensitive response elicitor protein or polypeptide. Moreover, from all of the foregoing, one of skill in the art would also have expected that such treatment would allow for both maximizing the benefit of the transgenic trait and overcoming any yield penalty associated therewith.

In the outstanding office action, the PTO cites to Dong et al., "Harpin Induces Disease Resistance in Arabidopsis through the Systemic Acquired Resistance Pathway Mediated by Salicylic Acid and the *NIM1* Gene," Plant J. 20:207-215 ("Dong") as evidence of one instance in which the application of a hypersensitive response elicitor protein or polypeptide did not overcome the deleterious effect of a transgene in a transgenic plant. Applicants submit that Dong is irrelevant to the presently claimed invention. In particular, the transformation of Arabidopsis plants with the transgene *nahG* did not confer a value-added trait, nor was it intended to confer a value-added trait to transgenic Arabidopsis plants. To the contrary, the transformation of Arabidopsis with the transgene *nahG* was intended only to prevent the accumulation a salicylic acid and thereby inactivate biochemical pathways associated with disease resistance. Infiltration of *nahG*-transgenic Arabidopsis with HrpN protein was conducted in order to elucidate parts of the signal transduction pathways that lead to systemic acquired resistance in plants (page 208, left column, ¶2), not as a means to overcome the deleterious effect of a value-added trait conferred by a transgene in a transgenic plant. Because Dong is not relevant to the presently claimed invention, it does not support the rejection put forth by the PTO.

For all these reasons, applicants submit that the invention of claims 1-21 is fully enabled by the disclosure of the present application, coupled with the knowledge in the art at the time the present application was filed. Therefore, the rejection of claims 1-21 for lack of enablement is improper and should be withdrawn.

The rejection of claims 1-21 under 35 U.S.C. § 112 (second paragraph) for indefiniteness have been overcome by the amendments and should therefore be withdrawn.

The rejection of claims 1-10 under 35 U.S.C. § 102(b) as being anticipated by PCT Application WO 98/24297 to Qiu et al. ("Qiu") is respectfully traversed. Qiu discloses imparting disease resistance to a plant transformed with a transgene encoding a hypersensitive response elicitor protein or polypeptide and topically applying a hypersensitive response elicitor to such a transgenic plant. As noted above, the present application clearly states at page 33, lines 10-12, that the transgene conferring a value-added trait does not encode a hypersensitive response elicitor protein or polypeptide. Claims 75 and 76 explicitly recite such a negative limitation. Because Qiu does not teach or suggest topical application of the hypersensitive response elicitor to a transgenic plant or plant seed as claimed (i.e., possessing a transgene that confers a value added trait but does not encode a hypersensitive response elicitor), Qiu cannot anticipate the presently claimed invention. Therefore, the rejection of claims 1-10 is improper and should be withdrawn.

The rejection of claims 1-10 under 35 U.S.C. § 102(b) as being anticipated by PCT Application WO 99/07206 to Bogdanove et al. ("Bogdanove") is respectfully traversed. Bogdanove, like Qiu, discloses imparting disease resistance to a plant transformed with a transgene encoding a hypersensitive response elicitor protein or polypeptide and topically applying a hypersensitive response elicitor to such a transgenic plant. As noted above, independent claims 75 and 76 explicitly recite that the transgene does not encode a hypersensitive response elicitor protein or polypeptide. Because Bogdanove does not teach or suggest topical application of the hypersensitive response elicitor to a transgenic plant or plant seed as claimed (i.e., possessing a transgene that confers a value added trait but does not encode a hypersensitive response elicitor), Bogdanove cannot anticipate the presently claimed invention. Therefore, the rejection of claims 1-10 is improper and should be withdrawn.

The rejection of claims 1-14, 17-18 and 20-21 under 35 U.S.C. § 102(b) as being anticipated by Keller et al., "Pathogen-Induced Elicitin Production in Transgenic Tobacco Generates a Hypersensitive Response and Nonspecific Disease Resistance," Plant Cell 11:223-235 (1999) ("Keller") is respectfully traversed.

Keller reports the production of transgenic plants that were transformed with a transgene containing a pathogen-inducible promoter fused to a gene encoding the protein cryptogein from *Phytophthora cryptogea* (page 223, abstract). Keller recites that cryptogein belongs to the family of elicitors referred to as "elicitins" that are produced in most *Phytophthora* species (page 224, left column, ¶2). *Phytophthora parasitica* var *nicotianea*, the causal agent of tobacco black shank disease, is an exception to this generalization and does not produce cryptogein or any other elicitin protein (Id.). Keller reports infiltrating cryptogein-transgenic tobacco plants with *Phytophthora parasitica* var *nicotianea* and observing a hypersensitive response that is typically only observed in tobacco plants infected by pathogens that produce elicitin proteins (page 225, right column, ¶2).

Applicants respectfully submit that the PTO has misconstrued the teachings of Keller.

The PTO asserts at page 9 of the outstanding office action that "the transgenic trait on the transgene is associated with a deleterious effect on disease resistance because these plants are not resistant to *Erysiphe cichoracearum*." Applicants respectfully disagree, because the lack of resistance to *Erysiphe cichoracearum*, or to *Thielaviopsis basicola* for that matter, in the cryptogein-transgenic plants is not a result of the transformation of the transgene into the plant. As clearly demonstrated in Table 1 and Table 2 of Keller, the wild type tobacco variety, the same tobacco variety transformed with the cryptogein transgene, also was not resistant to both *Erysiphe cichoracearum* and *Thielaviopsis basicola*. As such, the lack of disease resistance to both *Erysiphe cichoracearum* and *Thielaviopsis basicola* was present in the tobacco line prior to transformation and therefore not a result of the transformation with the transgene. The cited lack of disease resistance cannot be considered a deleterious effect caused by the transgene.

At page 9 of the office action, the PTO also asserts that "Keller et al., teach a method of applying *Phytophthora parasitica* var. *nicotianae*, which comprises the hypersensitive response elicitor protein cryptogein . . . to these plants (citation omitted)". While Keller clearly teaches challenging the transgenic plants with *P. parasitica* var. *nicotianae*, this pathogen is identified by Keller as exceptional in that it does not produce

elicitins (page 224, left column, ¶ 2). Applicants do acknowledge, however, that Keller teaches topical application of other *Phytophthora* species as well as harpin$_{Pss}$.

Keller, like Bogdanove and Qiu, discloses topical application of a hypersensitive response elicitor, harpin$_{Pss}$, but to a transgenic plant containing a transgene encoding the elicitin cryptogein. The elicitin cryptogein, like the class of art-recognized hypersensitive response elicitors described above, also elicits a hypersensitive response. As noted above, independent claims 75 and 76 explicitly recite that the transgene does not encode a hypersensitive response elicitor protein or polypeptide. Because Keller does not teach or suggest topical application of the hypersensitive response elicitor to a transgenic plant or plant seed as claimed (i.e., possessing a transgene that confers a value added trait but does not encode a hypersensitive response elicitor), Keller cannot anticipate the presently claimed invention. Therefore, the rejection of claims 1-14, 17-18, and 20-21 over Keller is improper and should be withdrawn.

The rejection of claims 1-21 under 35 U.S.C. § 103(a) as being unpatentable over each of Qui and Bogdanove is respectfully traversed. For substantially the same reasons as noted above, applicants submit that the presently claimed invention would not have been obvious over Qiu or Bogdanove, either alone or in combination. Independent claims 75 and 76 explicitly recite that the transgene (in the plant or plant seed) is one which "confers a value-added transgenic trait . . . but does not encode a hypersensitive response elicitor protein or polypeptide." Neither Qiu nor Bogdanove teach or suggest this limitation of the presently claimed invention. For this reason, the rejection is improper and therefore should be withdrawn.

In view of the all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: April 7, 2003

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: U.S. Patent and Trademark Office P.O. BOX 2327 Arlington, VA 22202, on the date below.
Date 4/7/03 Jane C. Wirszyla

APPENDIX
Version With Markings to Sh w Changes Made
Page 1 of 2

In reference to the amendments made herein to the claims, additions appear as underlined text while deletions appear as strikeout text, as indicated below:

In the Claims:

3. (Amended) The method according to claim ~~2~~ 75, wherein said applying is carried out on a plant.

4. (Amended) The method according to claim 3, wherein said applying is carried out by spraying, injection, or dusting~~, or leaf abrasion at a time proximate to when said applying takes place~~.

5. (Amended) The method according to claim ~~2~~ 75, wherein said applying is carried out on a plant seed.

7. (Amended) The method according to claim ~~2~~ 75, wherein the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed as a composition further comprising a carrier.

10. (Amended) The method according to claim ~~2~~ 75, wherein the hypersensitive response elicitor polypeptide or protein is in isolated form.

11. (Amended) The method according to claim ~~2~~ 75, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

13. (Amended) The method according to claim ~~12~~ 76, wherein said applying is carried out on a plant.

14. (Amended) The method according to claim 13, wherein said applying is carried out by spraying, injection, or dusting~~, or leaf abrasion at a time proximate to when said applying takes place~~.

15. (Amended) The method according to claim ~~12~~ 76, wherein said applying is carried out on a plant seed.

17. (Amended) The method according to claim ~~12~~ 76, wherein the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed as a composition further comprising a carrier.

20. (Amended) The method according to claim ~~12~~ 76, wherein the hypersensitive response elicitor polypeptide or protein is in isolated form.

21. (Amended) The method according to claim ~~12~~ 76, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

#18 Affidavit

PATENT
Docket No. **21829/91 (EBC-007)**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Wei et al.	Examiner: A. Kubelik
Serial No.	:	09/880,371	Art Unit: 1638
Cnfrm. No.	:	4973	
Filed	:	June 13, 2001	
For	:	METHODS OF IMPROVING THE EFFECTIVENESS OF TRANSGENIC PLANTS	

DECLARATION OF ERNEST J. DE ROCHER UNDER 37 C.F.R. § 1.132

U.S. Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

I, ERNEST J. DE ROCHER, pursuant of 37 C.F.R. § 1.132, declare:

1. I received a B.S. degree in Biology from the University of Washington, Seattle, Washington in 1985, and a Ph.D. degree in Molecular and Cellular Biology from the University of Arizona, Tucson, Arizona in 1992.

2. I am currently employed as a Senior Scientist at the EDEN Bioscience Corporation in Bothell, Washington.

3. I am an inventor of the above-identified application.

4. I am presenting this declaration to show that hypersensitive response elicitors from a diverse range of plant pathogenic bacteria (1) are an art-recognized class of proteins where results achieved with one such protein would be expected when other proteins in this class are used and (2) share the unique ability to cause distinct plant responses through the topical application of the protein on a plant. In addition, I am presenting this declaration to specifically show that the application of hypersensitive response elicitors, from a diverse range of plant pathogenic bacteria, to transgenic plants or plants grown from transgenic plant seeds increases the efficacy and thereby maximizes the benefit of value added traits conferred by transgenes in transgenic plants or plants grown from transgenic plant seeds, in comparison to transgenic plants or plants grown from transgenic plant seeds to which no hypersensitive response elicitor has been applied. I am also presenting this declaration to provide

evidentiary support of the fact that some value-added traits are associated with a yield penalty.

5. In plants, the hypersensitive response phenomenon results from an incompatible interaction between plant pathogens and non-host plants. As explained in Gopalan et al., "Bacterial Genes Involved in the Elicitation of Hypersensitive Response and Pathogenesis," Plant Disease 80: 604-10 (1996) ("Gopalan") (attached hereto as **Exhibit 1**), these types of interactions involve, for example, a bacterial plant pathogen attempting to infect a host plant, and the host plant preventing proliferation of the pathogen by the collapse and death, or necrosis, of plant leaf cells at the site of infection. This is distinct from a compatible interaction between a bacterial plant pathogen and a host plant in which the bacteria is capable of proliferation, resulting in the spread of the pathogen throughout the plant and the manifestation of disease symptoms. Id. at 604.

6. Hypersensitive response elicitors within a given genus are often homologous to elicitors from different pathogenic species and strains of the same genus. For example, homologs of hypersensitive response elicitors from *Erwinia amylovora* and *Pseudomonas syringae* have been identified in different bacteria species and strains from the genera *Erwinia* and *Pseudomonas*, respectively. See Gopalan.

7. In addition, numerous reported studies confirm that a gene encoding a hypersensitive response elicitor from a particular source genus can be used to isolate a corresponding hypersensitive response elicitor gene from different species and strains of that same genus. For example, in Bauer et al., "*Erwinia chrysanthemi* $\text{Harpin}_{\text{Ech}}$: An Elicitor of the Hypersensitive Response that Contributes to Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995) ("Bauer") (attached hereto as **Exhibit 2**), the *Erwinia amylovora* hypersensitive response elicitor encoding gene was used as a probe to isolate, clone, and sequence the gene encoding the *Erwinia chrysanthemi* hypersensitive response elicitor, as follows:

> The cosmids were probed in colony blots with a 1.3-kb *Hind*III fragment from pCPP1084, which contains the *E. amylovora hrpN* gene (Wei et al. [, "Harpin Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (]1992[)]). pCPP2157, one of the three cosmids hybridizing with the probe, was digested with several restriction enzymes, and the location of the $hrpN_{Ech}$ gene in those fragments was determined by probing a Southern blot with *E. amylovora Hind*III fragment. Two fragments, each containing the entire $hrpN_{Ech}$ gene, were subcloned into different vectors: pCPP2142 contained an 8.3-kb *Sal*I fragment in pUC119 (Vieira and Messing [,"Production of Single-Stranded Plasmid DNA," Methods Enzymol., 153:3-11(]

> 1987[])], and pCPP2141 contained a 3.1-kb *Pst*I fragment in pBluescript II SK(-) (Stratagene, La Jolla, CA).
>
> ***Sequence of hrpN$_{Ech}$***
>
> The nucleotide sequence of a 2.4-kb region of pCPP2141 encompassing *hrpN$_{Ech}$* was determined. The portion of that sequence extending from the putative ribosome-binding site through the *hrpN$_{Ech}$* coding sequence to a putative rho-independent terminator is presented in Figure 1.

See page 485.

8. In the same manner as described in Bauer *supra*, Cui et al., "The RsmA$^-$ Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp*N$_{Ecc}$ and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996) ("Cui") (attached hereto as **Exhibit 3**) demonstrates that the gene encoding the *Erwinia carotovora* hypersensitive response elicitor can be isolated, sequenced, and cloned by using the *Erwinia chrysanthemi* hypersensitive response elicitor encoding gene to probe the genomic library of *Erwinia carotovora*. Further, Cui (at page 572) states the following:

> The genomic library of *E. carotovora* subsp. *carotovora* strain Ecc71 in pLARF5 was screened by in situ colony hybridization with a 0.75-kb internal *Cla*I fragment of *hrpN* of *E. chrysanthemi* (Bauer et al.[, "*Erwinia chrysanthemi* Harpin$_{Ech}$: An Elicitor of the Hypersensitive Response that Contributes to Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (]1995). Two cosmids, pAKC921 and pAKC922, that hybridized with the probe were isolated. The subclones (pAKC923 and pAKC924, Table 1) carrying *hrpN* DNA were used for sequence analysis.

9. The gene encoding the hypersensitive response elicitor of *Erwinia amylovora* has also been used as a probe to isolate and clone the gene encoding the hypersensitive response elicitor of *Erwinia stewartii*. It was found that antibodies raised against the hypersensitive response elicitor of *Erwinia stewartii* cross-reacted with the hypersensitive response elicitor of *Erwinia amylovora*. See Ahmad et al., "Harpin Is Not Necessary for the Pathogenicity of Maize," 8th Int'l Cong. Molec. Plant Microbe Inter. July 14-19, 1996 ("Ahmad") (attached hereto as **Exhibit 4**).

10. Similar findings were reported for hypersensitive response elicitors from the genus *Pseudomonas*. An internal fragment of the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae* (i.e., *hrpZ*) was used to identify and isolate the hypersensitive response elicitors from *P. syringae* pv. *glycinea* and *P. syringae* pv. *tomato*. Significant amino acid sequence similarities were identified between the various

Pseudomonas syringae elicitors. See Preston et al., "The HrpZ Proteins of *Pseudomonas syringae* pvs. *syringae, glycinea,* and *tomato* Are Encoded by an Operon Containing *Yersinia ysc* Homologs and Elicit the Hypersensitive Response in Tomato But Not Soybean," MPMI 8(5): 717-32 (1995) ("Preston") (attached hereto as **Exhibit 5**).

11. The genes encoding hypersensitive response elicitors are positioned within the *hrp* gene cluster or proximate to the *hrp* gene cluster in *hrp* regulons. For example, *hrpN* from *Erwinia amylovora* was located within the *hrp* gene cluster, as was *hrpZ* from *Pseudomonas syringae.* The *popA* gene, encoding a hypersensitive response elicitor from *Pseudomonas* (now Ralstonia) *solanacearum*, was located on the left flank of the *hrp* gene cluster within a *hrp* regulon. See Bonas, "*hrp* Genes of Phytopathogneic Bacteria," Current Topics in Microbiology and Immunology 192: 79-98 (1994) ("Bonas I") (attached hereto as **Exhibit 6**) and Alfano et al., "The Type III (Hrp) Secretion Pathway of Plant Pathogenic Bacteria: Trafficking Harpins, Avr Proteins, and Death," Journal of Bacteriology 179: 5655-5662 (1997) ("Alfano") (attached hereto as **Exhibit 7**). Similar to the *popA* gene, *hreX*, the gene encoding a hypersensitive response elicitor from *Xanthomonas campestris*, was located on the left flank of the *hrp* gene cluster. See Swanson et al., "Isolation of the *hreX* Gene Encoding the HR Elicitor Harpin (Xcp) from *Xanthomonas Campestris* pv. *pelargonii*," Phytopathology 90: s75 (1999) ("Swanson") (attached hereto as **Exhibit 8**).

12. The characteristics that distinguish hypersensitive response elicitors as a distinct class of molecules are clearly apparent when considering the different elicitors' secretion mechanisms, regulation, biochemical characteristics, and biological activities.

13. Substantially all hypersensitive response elicitors identified have been shown to be secreted through the type III, *hrp* dependent secretion pathway. The type III secretion pathway is a highly conserved and unique mechanism for the delivery of pathogenicity related molecules in gram-negative bacteria. The *hrp* gene cluster is largely composed of components of the type III secretion system. See Bogdanove et al., "Unified Nomenclature for Broadly Conserved *hrp* Genes of Phytopathogenic Bacteria," Molec. Microbiol. 20:681-83 (1996) ("Bogdanove") (attached hereto as **Exhibit 9**); and Alfano.

14. Regulation of the genes encoding the *hrp* gene cluster, and subsequently the genes encoding the components of the type III secretion system and hypersensitive response elicitors, is controlled by environmental factors. Specifically, transcriptional expression of these genes is induced under conditions that mimic the plant apoplast, such as low concentrations of carbon and nitrogen, low temperature, and low pH. See Wei et al., "Regulation of hrp Genes and Type III Protein Secretion in *Erwinia*

amylovora by HrpX/HrpY, a Novel Two-Component System, and HrpS," MPMI 13(11): 1251-1262 (2000) ("Wei I") (attached hereto as **Exhibit 10**); and Bonas I.

15. Biochemically, hypersensitive response elicitors have a number of common characteristics. These include being glycine rich, heat stable, hydrophilic, lacking of an N-terminal signal sequence, and susceptible to proteolysis. See Bonas, "Bacterial Home Goal by Harpins," Trends Microbiol 2: 1-2 (1994) ("Bonas II") (attached hereto as **Exhibit 11**); Bonas I; Gopalan; and Alfano.

16. In addition, hypersensitive response elicitors share a unique secondary structure that has been associated with these elicitors' distinct biological activities (described below). The structure has two primary components, an alpha helix unit and a relaxed acidic unit having a sheet or random turn structure. In the absence of one or both of these components, hypersensitive response elicitation does not occur. See WO 01/98501 to Fan et al. ("Fan") (attached hereto as **Exhibit 12**).

17. In addition to eliciting the hypersensitive response in a broad range of plant species, as explained by Wei et al., "Harpin from *Erwinia amylovora* Induced Plant Resistance," Acta Horticulture 411: 223-225 (1996) ("Wei II") (attached hereto as **Exhibit 13**) and by Alfano, hypersensitive response elicitors also share the ability to induce specific plant responses. The induction of plant disease resistance, plant growth enhancement, and plant stress resistance are three plant responses that result from treatment of plants or plant seeds with a hypersensitive response elicitor from a gram-negative plant pathogen.

18. As described in Wei II, treatment of plants with the hypersensitive response elicitor HrpN from *Erwinia amylovora* resulted in disease resistance to a broad range of plant pathogens. For example, HrpN induced disease resistance to southern bacterial wilt (*Pseudomonas solanacearum*) in tomato, tobacco mosaic virus in tobacco, and bacterial leaf spot (*Gliocladium cucurbitae*) in cucumber.

19. The hypersensitive response elicitor HrpZ from *Pseudomonas syringae* was reported to induce disease resistance in cucumber to a diverse range of pathogens, including the fungal disease *Colletotrichum lagenarium*, tobacco necrosis virus, and bacterial angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*). See Strobel et al., "Induction of Systemic Acquired Resistance in Cucumber by *Pseudomonas syringae* pv. *syringae* 61 $HrpZ_{Pss}$ Protein," Plant Journal 9(4): 431-439 (1996) ("Strobel") (attached hereto as **Exhibit 14**).

20. Hypersensitive response elicitors from *Erwinia amylovora* and *Pseudomonas syringae* pv. *syringae* are also known to enhance plant growth. See Examples

1 to 24 of U.S. Patent No. 6,277,814 to Qiu et al. ("Qiu") (attached hereto as **Exhibit 15**), which showed that treatment of plants and plant seeds with HrpN from *E. amylovora* induced plant growth enhancement in species of tomato, potato, raspberry, and cucumber.

21. The hypersensitive response elicitor HrpN from *Erwinia amylovora* is known to induce desiccation resistance to cuttings removed from ornamental flowers. See Example 1 of WO 02/37960 to Wei et al. (attached hereto as **Exhibit 16**), which shows that postharvest application of HrpN to cut roses contributed to substantially greater longevity of the cut roses and that preharvest application of HrpN to cut roses contributed to a substantially greater increase in the number of flowers open at harvest.

Additional Experimental Evidence Showing That Hypersensitive Response Elicitors Induce Plant Disease Resistance

22. As demonstrated by the following experimental evidence in paragraphs 23 and 24 below, treatment of tomato and tobacco plants with the hypersensitive response elicitor HreX from *Xanthomonas campestris* induced disease resistance in the plants against bacterial wilt and tobacco mosaic virus.

23. The induction of disease resistance in tomato against bacterial wilt (caused by the pathogenic bacterium *Pseudomonas solanacearum* K_{60}) was investigated as follows. Approximately 30 days after sowing, tomato plants were sprayed with either a dilution of HreX or 5 mM potassium phosphate buffer, pH 6.8 (the same buffer used to dilute the HreX solution). Six days after treatment, inoculation was performed by slicing the soil of the pot containing the tomato plant 4 times and applying 40 ml of solution containing 1×10^6 colony forming units ("cfu") per ml of *P. solanacearum* K_{60} to the soil. Disease severity ratings were recorded at 7, 9, and 13 days after inoculation ("DAI"), as shown below in Table 1. As these results demonstrate, plants treated with HreX exhibited substantially more disease resistance than the buffer-treated control plants.

Table 1. *Pseudomonas solanacearum* Disease Resistance from Treatment of Tomato with HreX.

Treatment Groups[a]	Disease Index (7 DAI)	Disease Index (9 DAI)	Disease Index (12 DAI)	% Difference (12 DAI)
HreX	0.12	0.22	0.22	38.89
Buffer	0.16	0.3	0.36	na

[a]Each group consisted of 1 pot containing 10 plants.

24. Experiments examining the induction of systemic disease resistance in tobacco from treatment with HreX were conducted as follows: Diluted HreX was sprayed on all but the bottom most full-sized leaf of six- to eight-week-old tobacco plants (Xanthi NN). The bottom most full-sized leaf was covered during spraying so as not to receive residual spray. Three days after the spray treatment, the unsprayed leaf and the leaf opposite it, were lightly dusted with diatomaceous earth. Thereafter, 20 µl of a 1.7 µg/ml solution of tobacco mosaic virus ("TMV") was applied to both leaves dusted with diatomaceous earth. The TMV was gently and evenly spread across the leaves. Approximately 5 minutes after inoculation, the plants were lightly rinsed to remove the diatomaceous earth. Three days after inoculation, the number of TMV lesions on the unsprayed and sprayed leaves for each plant was recorded, as shown below in Table 2. As these results demonstrate, plants treated with HreX exhibited substantially more disease resistance than the buffer-treated control plants.

Table 2. Tobacco Mosaic Virus Resistance in Tobacco from Treatment with HreX.

Treatment Groups	Number of TMV Lesions on Leaf									
	Treated leaves					Untreated leaves				
	Plant No. 1	Plant No. 2	Plant No. 3	Avg. No.	% Difference	Plant No. 1	Plant No. 2	Plant No. 3	Avg. No.	% Difference
HreX	5	7	8	6.67a	93.37	41	22	20	27.67a	76.49
Buffer Control	107	99	96	100.67b	na	124	106	123	117.67b	na

Additional Experimental Evidence Showing Enhanced Plant Growth by Treatment of Plants with Hypersensitive Response Elicitor

25. As demonstrated by the following experimental evidence in paragraphs 26 and 27 below, treatment of plants with hypersensitive response elicitors from a range of sources, such as *Pseudomonas syringae* (HrpZ) and *Xanthomonas campestris* (HreX), enhances plant growth.

26. The hypersensitive response elicitor HreX from *Xanthomonas campestris* was evaluated for induction of plant growth enhancement as follows. Prior to sowing, tomato seeds were soaked for approximately four hours in either a solution containing the partially purified HreX protein diluted in potassium-phosphate buffer, or potassium-phosphate buffer alone. The treated seeds were then planted and maintained in identical conditions in a controlled environment. Each treatment group consisted of 3 pots, each pot containing 8 plants. The average plant heights and percent differences between the

treatment groups are shown below in Table 3. As these results demonstrate, plants treated with HreX grew significantly more than the buffer-treated control plants.

Table 3. Growth Enhancement from Treatment of Tomato with the Hypersensitive Response Elicitor HreX.

Treatment Groups	Replicates[1]			Mean[2]	% Difference
	Pot #1	Pot #2	Pot #3		
HreX	7.4	7.3	6.8	7.1a	15.5
Buffer Control	6.1	6.1	5.6	6.0b	na

[1] Mean height of the 8 plants in each pot.
[2] Means followed by the same letter do not significantly different (P=0.01, LSD)

27. The hypersensitive response elicitor HrpZ from *Pseudomonas syringae* was evaluated for induction of plant growth enhancement as follows. Prior to sowing, tomato seeds were soaked for approximately four hours in either a solution containing the partially purified HrpZ protein diluted in potassium-phosphate buffer, or potassium-phosphate buffer alone. The treated seeds were then planted and maintained in identical conditions in a controlled environment. Each treatment group consisted of 6 pots, each pot containing 10 plants. The average plant heights and percent differences between the treatment groups are shown below in Table 4. As these results demonstrate, plants treated with HrpZ grew significantly more than the buffer-treated control plants.

Table 4. Growth Enhancement from Treatment of Tomato with the Hypersensitive Response Elicitor HrpZ.

Treatment Groups	Replicates[1]						Mean[2]	% Difference
	Pot #1	Pot #2	Pot #3	Pot #4	Pot #5	Pot #6		
HrpZ	5.10	5.28	4.60	4.72	4.71	4.87	4.88a	9.6
Buffer Control	4.15	4.38	3.84	4.31	4.62	5.18	4.41b	na

[1] Mean height of the 18 to 21 plants in each pot.
[2] Means followed by the same letter do not significantly differ (P=0.054, LSD)

Additional Experimental Evidence Showing That Hypersensitive Response Elicitors Induce Plant Stress Resistance

28. As evidenced by the experimental results reported in Example 12 of Qiu and Examples 1-6 of WO 00/28055 to Wei et al. (attached hereto as **Exhibit 17**), HrpN

from *Erwinia amylovora* is capable of inducing various forms of plant stress resistance, such as chemical stress resistance, drought stress resistance, and nutritional stress resistance.

29. As demonstrated by the following experimental evidence in paragraphs 30-34 below, treatment of plant seeds with a hypersensitive response elicitor, from a diverse range of sources, can impart salt stress resistance plants grown from treated seeds.

30. In order to investigate whether treatment of plants with the hypersensitive response elicitor HrpN from *Erwinia amylovora* imparts salt stress resistance in plants, lima bean seeds (Dixie Speckled Peas) were treated with Messenger®, containing 3% active ingredient HrpN from *Erwinia amylovora* (EDEN Bioscience Corporation, Bothell, WA), sown, and then maintained in the presence of varying concentrations of NaCl. Lima beans were grown in pots containing vermiculite and equal amounts of the fertilizer EcoGrow (ECO Enterprises, Shoreline, WA). The treatment groups are as detailed below in Tables 5 and 6. Fifteen seeds were planted in each pot. Messenger treatments consisted of soaking the seeds in 100 ml of a solution containing 20 µg/ml of Messenger® dissolved in water. The seeds were soaked for approximately 4 hours at 26°C. Plants were grown at 22°C to 26°C with a 14 hour daylight period.

31. Results were obtained by measuring plant height and dry root weight for all plants that germinated. The roots were dried by cutting the root mass from the shoot and drying the root mass overnight at 26°C. The results are described below in Tables 5 and 6.

Table 5. Effect of Messenger® Treatments on Plant Height

Treatment	Mean Height(cm)	% Difference from UTC
NaCl 100 mM	5.76	-11.69
NaCl 200 mM	2.00	-69.35
NaCl 300 mM	0.57	-91.24
NaCl 100 + Messenger®	6.54	0.16
NaCl 200 + Messenger®	3.19	-51.06
NaCl 300 + Messenger®	.58	-91.06
Messenger®	7.26	11.30
UTC (untreated control)	6.53	0.00

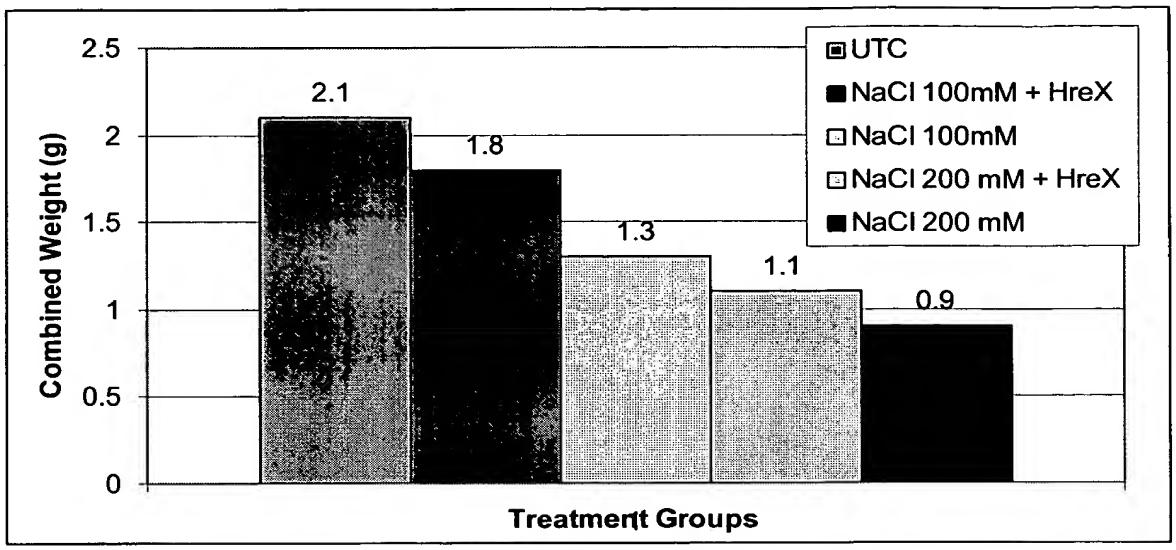
Table 6. Effect of Messenger® Treatments on Root Weight

Treatment	Mean Weight (g)	% Difference from UTC
NaCl 100 mM	0.22	-35.47
NaCl 200 mM	0.11	-68.97
NaCl 300 mM	0.02	-93.10
NaCl 100 + Messenger®	0.26	-22.17
NaCl 200 + Messenger®	0.14	-57.64
NaCl 300 + Messenger®	0.04	-89.16
Messenger®	0.38	11.33
UTC (untreated control)	0.34	0.00

32. The ability of Messenger® to impart salt stress resistance on plants is clearly demonstrated by the results set forth in Tables 5 and 6, where salt-induced reductions in plant height and root weight are lessened with Messenger®. In particular, at 200 mM NaCl, Messenger® treated plants had an increase in root weight of approximately 11 percent and an increase in plant height of approximately 18 percent over that of the non-Messenger® treated plant. At 100 mM NaCl, Messenger® treated plants had an increase in root weight of approximately 13 percent and an increase in height of approximately 12 percent over that of the non-Messenger® treated plant.

33. In order to investigate whether treatment of plants with the hypersensitive response elicitor HreX from *Xanthomonas campestris* imparts salt stress resistance in plants, lima bean seeds (Dixie Speckled Peas) were treated with HreX, sown, and then maintained in the presence of varying concentrations of NaCl. HreX treatment consisted of soaking the seeds in 100 ml of a solution containing a 3% formulation of HreX dissolved in water. The seeds were soaked for approximately 4 hours at 26°C. The lima beans were grown in pots containing vermiculite, equal amounts of the fertilizer EcoGrow (ECO Enterprises, Shoreline, WA), and varying concentrations of NaCl. The treatment groups were as detailed below in Figure 1. Fifteen seeds were planted in each pot, with a total of six pots per treatment. Plants were grown at 22°C to 26°C with a 14 hour daylight period. Results were obtained by measuring the dry weight from the largest 10 plants from each pot. The plants were dried by isolating the entire plant from the vermiculate and drying overnight at 26°C. The Combined Weights detailed in Figure 1 below represent the accumulated dry weight of the 60 plants measured from each treatment. The untreated control (UTC) plants were not treated with HreX and were not grown in the presence of NaCl. The results of the study are shown below in Figure 1.

Figure 1. Dry Weight of Salt + HreX and Salt Alone Treated Plants



34. The hypersensitive response elicitor HreX clearly imparts salt stress resistance in plants, as demonstrated in Figure 1. Growth of the plants in the presence of 100 mM and 200 mM NaCl resulted in decreases in plant dry weight of approximately 38% and 57%, respectively, in comparison to that of the untreated control plants. In contrast, plants treated with HreX and grown in the presence of 100 mM and 200 mM NaCl resulted in decreases in plant dry weight of approximately 14% and 48%, respectively, in comparison to that of the untreated control plants. The treatment of plants growing in the presence of high concentrations of NaCl with the hypersensitive response elicitor HreX resulted in increases in plant dry weight of 18 to 28%.

Additional Experimental Evidence Showing Application of Hypersensitive Response Elicitors to Transgenic Plants and Plants Grown from Transgenic Plant Seeds Maximizes the Benefit of a Transgenic Plant's Value Added Trait.

35. As demonstrated by the following experimental evidence in paragraphs 36-38 below, hypersensitive response elicitors, from a diverse range of plant pathogenic bacteria, are capable of maximizing the benefit of value added traits in transgenic plants and plants grown from transgenic plant seeds.

36. A Roundup® resistant transgenic corn variety was sown using conventional field corn production techniques in randomized blocks of approximately 7.5 feet in width and 30 feet in length. There were four rows of corn planted per block. All four rows were treated as described below. The two center rows of each plot were used for data

collection. The following treatment groups were included in the study: (1) Roundup UltraMAX® at 52 oz. per acre (active ingredient glyphosate, Monsanto, St. Louis, MO) (RU 2X), (2) Roundup UltraMAX® at 52 oz. per acre + Messenger® at 2.25 oz. per acre (active ingredient HrpN protein from *Erwinia amylovora*, EDEN Bioscience Corporation, Bothell, WA) (RU 2X + Messenger®), (3) Roundup UltraMAX® at 104 oz. per acre (RU 4X), (4) Roundup UltraMAX® at 104 oz. per acre + Messenger® at 2.25 oz. per acre(RU 4X + Messenger®), and (5) an un-treated control (UTC) with no applications of either Roundup UltraMAX® or Messenger®. Table 7 below shows the yield differences between the different treatment groups. Application of Roundup UltraMAX® to Roundup® resistant corn resulted in a 12% to 13% decrease in the yield in comparison to the UTC. Inclusion of Messenger® in the treatment group increased the Roundup® resistant corn's resistance to Roundup® and resulted in only 1% to 4% decreases in yield in comparison to the UTC. Increased resistance to Roundup UltraMAX® was also observed in the overall plant height, as seen below in Table 8. There were no significant differences observed between the plant heights of the UTC and Roundup UltraMAX® + Messenger® treatment groups. In contrast, application of Roundup® UltraMAX® alone resulted in significantly lower plant heights, approximately 9% lower than the UTC® and Roundup UltraMAX® + Messenger® treatment groups.

Table 7: Yield of Roundup® and Roundup® + Messenger® treated Roundup® Resistant Transgenic Corn.

Treatment Group	Mean Yield (Bu/acre)	% Difference from UTC
UTC	100.6	NA
RU 2X	87.9	-12.6
RU 2X + Messenger	98.8	-1.2
RU 4X	87.1	-13.4
RU 4X + Messenger	96.2	-3.8

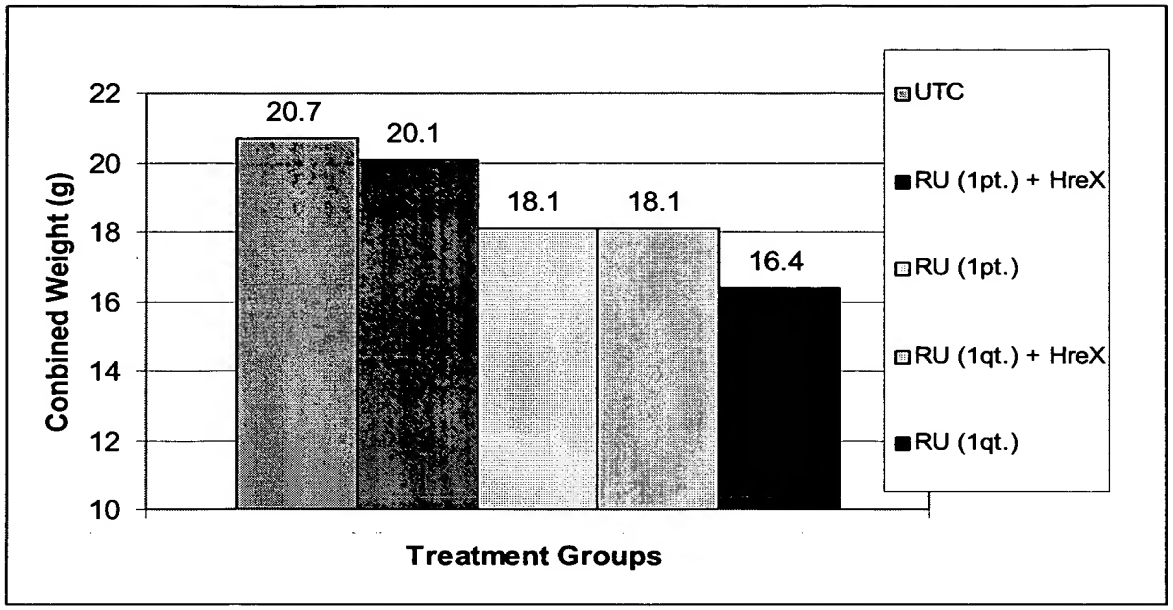
Table 8: Plant Height in Roundup® and Roundup® + Messenger® treated Roundup® Resistant Transgenic C rn.

Treatment Group	Mean Plant Height (inches)
UTC	73.5a
RU 2X	67.0b
RU 2X + Messenger	73.8a
RU 4X	66.8b
RU 4X + Messenger	73.3a

Means followed by the same letter do not significantly differ (P=0.05, LSD)

37. A Roundup® herbicide resistant transgenic corn seed was treated with HreX, a hypersensitive response elicitor protein from *Xanthomonas camperstris*, and then treated with varying concentrations of Roundup® (active ingredient glyphosate, Monsanto Co., St. Louis, MO). The HreX treatments involved soaking the corn seeds in 100 ml of a solution containing a 3% formulation of HreX dissolved in water. The seeds were soaked for approximately 4 hours at 26°C. The corn seeds were sown in pots containing vermiculite and equal amounts of the fertilizer EcoGrow™ (ECO Enterprises, Shoreline, WA). Roundup® (RU) treatments were conducted by a single spraying of the corn seedlings approximately two weeks after germination. Roundup® was applied at two concentrations. At the 1 pint (1pt.) application rate, 4.73 ml of Roundup® was mixed with 189 ml water. At the 1 quart (1qt.) application rate, 9.46 ml of Roundup® was mixed with 189 ml of water. The specific treatment groups were as detailed below in Figure 2. Fifteen seeds were planted in each pot with a total of six pots per treatment. Plants were grown at 22°C to 26°C with a 14 hour daylight period. Results were obtained by measuring the dry weight from the largest 10 plants from each pot. The plants were dried by isolating the entire plant from the vermiculate and drying overnight at 26°C. The Combined Weight shown below in Figure 2 represents the accumulated dry weight of the 60 plants measured from each treatment group. The untreated control (UTC) plants were not pretreated with HreX and were not treated with Roundup®.

Figure 2. Dry Weight of Chemical + HreX and Chemical Alone Treated Plants



38. The hypersensitive response elicitor HreX clearly imparts chemical stress resistance in plants as demonstrated in Figure 2. Treatment of plants with Roundup® led to decreases in plant dry weight of approximately 13% at the Roundup® application rate of 1 pint, and approximately 21% at the Roundup® application rate of 1 quart, in comparison to that of the untreated control plants. In contrast, plants treated with HreX in combination with Roundup® resulted in decreases in dry weight of approximately 3% at the Roundup® application rate of 1 pint, and approximately 13% at the Roundup® application rate of 1 quart, in comparison to that of the untreated control plants. The treatment of plants with the hypersensitive response elicitor HreX increased the transgenic plant's resistance to Roundup® by 9 to 10%.

Evidence of Deleterious Effects from Transgenes Conferring Value Added Traits in Transgenic Plants or Plants Grown from Transgenic Plant Seed

39. Transgenic glyphosate resistant soybean and non-transgenic soybean sister lines were evaluated in four locations over a two year period for differences in yields. The glyphosate resistant soybean lines yielded approximately 5% less (kg/ha) than the non-transgenic soybean sister lines. Accordingly, Elmore et al. "Glyphosate-Resistant Soybean Cultivars Yield Compared with Sister Lines," Agronomy Journal 93:408-412 (2001) (copy attached hereto as **Exhibit 18**), concluded that there was in fact a yield suppression in the transgenic soybean lines in comparison to the non-transgenic soybean sister lines.

40. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: April 7, 2003

Ernest J. De Rocher

Ernest J. De Rocher